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# The Neuregulin 1 Promoter Polymorphism rs6994992 is Not Associated with Chronic Schizophrenia or Neurocognition

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## Abstract

The neuregulin 1 (*NRG1*) promoter single nucleotide polymorphism (SNP) rs6994992 has shown association with decreased activation of frontal and temporal lobe regions, increased risk of psychosis, and decreased premorbid IQ. This SNP is part of a putative schizophrenia risk-associated haplotype and was associated with increased expression of the type IV transcript in postmortem tissue. We tested for association between rs6994992 and chronic schizophrenia by genotyping 738 cases from the Clinical Antipsychotic Trials of Intervention Effectiveness (CATIE) and 733 matched controls. We further tested for associations with age at onset and baseline neurocognition in cases with schizophrenia reasoning that these phenotypes might yield results similar to those seen for premorbid IQ. Affection status was weakly associated with rs6994992 genotypes and trended towards association under a recessive model. This association did not survive correction for multiple comparisons and was in the opposite direction than has been reported. There was no association between rs6994992 and age at onset, an estimate of premorbid IQ, or neurocognition at study baseline. We were unable to replicate previous associations of rs6994992 with schizophrenia and, moreover, did not find significant associations with age of onset, an estimate of pre-morbid IQ, or neurocognition.

### Keywords

schizophrenia; *NRG1*; rs6994992; neurocognition; association study

In a recent *Nature Neuroscience* paper, Hall and colleagues (Hall et al., 2006) investigated the effects of rs6994992, a neuregulin 1 (*NRG1*) promoter single nucleotide polymorphism (SNP), in Scottish subjects at high risk of schizophrenia by virtue of having at least two affected family members. They demonstrated significant association of this polymorphism with decreased activation of frontal and temporal lobe regions, increased risk of psychosis, and decreased premorbid IQ. The effect of this SNP was exceptional – 12 of 12 subjects with the TT genotype developed psychotic symptoms compared to 19/40 with CT and 12/27 with CC genotypes. Moreover, rs6994992 (also known as SNP8NRG243177) is part of a putative schizophrenia risk-associated haplotype (Li et al., 2006; Stefansson et al., 2003;

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Stefansson et al., 2002) that is predicted to alter the putative binding sites for three transcription factors and was associated with increased expression of the type IV transcript in postmortem tissue (Law et al., 2006). Given that the effect of rs6994992 was so marked, we tested for association between rs6994992 and chronic schizophrenia by genotyping 738 cases and 733 matched controls. We further tested for associations with age at onset and baseline neurocognition in cases with schizophrenia reasoning that these phenotypes might yield results similar to those of Hall et al (2006).

We genotyped rs6994992 (chr8:31,615,123, NCBI Build 35) in 738 participants (74% male, 26% female; 57% European ancestry, 29% African, 14% other) in CATIE (Clinical Antipsychotic Trials of Intervention Effectiveness), a double-blinded randomized clinical trial of typical and atypical antipsychotics under controlled conditions with follow-up for as long as 18 months, and in which all subjects provided informed consent under IRB approval (Lieberman et al., 2005). Briefly, inclusion criteria required a definite diagnosis of schizophrenia, previous antipsychotic treatment, age 18–65 years, clinical decision that oral medication was appropriate, adequate decisional capacity and provision of written informed consent. Exclusion criteria essentially stipulated that participation was safe and clinically reasonable (Stroup et al., 2003). The construction, implementation, and analyses of the neurocognitive data from CATIE are described at length elsewhere (Keefe et al., 2006; Keefe et al., 2003). Briefly, this battery assessed aspects of neurocognitive function that are robustly associated with schizophrenia, that could plausibly improve with treatment, and which were practical to do in a large patient sample treated in “real world” clinical care settings. We tested for association with two variables: WRAT-3 score (a proxy measure of premorbid IQ) (Griffin et al., 2002) and a composite neurocognitive score (Keefe et al., 2006).

There were 733 control subjects (67% male, 33% female; 56% European ancestry, 30% African, 14% other), group-matched to cases by age, sex, and self-reported race. Controls were ascertained from a US national sampling frame as part of the Gejman MGS study (MH059571). Controls were collected by Knowledge Networks (KN), a survey and market research company whose panel contains approximately 60,000 households (>120,000 unrelated adults) that were selected by random digit dialing. KN provided financial incentives to its panel members for participation in web-based surveys. The panel is representative of the US population (except for a slight bias toward higher income and education). Subjects were sampled proportionally from 25 major population areas. Exclusion criteria were self-reported history of psychotic or bipolar disorders. Written informed consent was obtained from all participants.

The SNP rs6994992 was genotyped using TaqMan (Livak 1999) blind to all phenotypic data and passed quality control – minor allele frequency agreed with published reports (Li et al., 2006), perfect agreement of 99 duplicated samples with originals, overall no-call proportion was 0.014, and genotype frequencies were consistent with Hardy-Weinberg equilibrium overall (p=0.48) and in the major ancestry groups (AFR p=0.49 and EUR p=0.48).

Association results are shown in the Table. Affection status was weakly associated with rs6994992 genotypes and trended towards association under a recessive model (TT versus CT/TT). This association did not survive correction for multiple comparisons and was in the opposite direction than has been reported (i.e., T allele less prevalent in cases) (Li et al., 2006). Within the schizophrenia sample, we saw no association between rs6994992 and age at onset, an estimate of premorbid IQ, or neurocognition at study baseline.

In conclusion, we were unable to replicate previous associations of rs6994992 with schizophrenia and, moreover, did not find significant associations with age of onset, an

estimate of pre-morbid IQ, or neurocognition. Our study had 80% power to detect a genotypic relative risk of 1.29 or larger (log additive model, allele frequency 0.4, and two-tailed p=0.01) (Gauderman 2002). It is important to note the dissimilarities of this sample and phenotypes to that of Hall et al (2006) as we studied individuals with chronic schizophrenia and used different phenotype measures.

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## Table

Lack of association between *NRG1* rs6994992 and schizophrenia, WRAT-3 scores, age of onset, and neurocognition.

| Association Test | Group | Genotype [a] | | | Association Test |
|---|---|---|---|---|---|
| | | C/C | C/T | T/T | |
| Schizophrenia | Cases | 263 (0.363) | 363 (0.507) | 99 (0.137) | $\chi_1^2$ =3.96, p=0.046 [b] |
| | Controls | 236 (0.325) | 366 (0.504) | 124 (0.171) | $\chi_1^2$ =3.20, p=0.074 [c] |
| Age of onset | Cases | 27.0 (0.62) | 26.9 (0.55) | 27.4 (0.95) | $t_1$ =0.21, p=0.84 [d] |
| WRAT-3 score (proxy for premorbid IQ) | Cases | 42.5 (0.60) | 42.2 (0.54) | 41.6 (0.91) | $t_1$ =−0.79, p=0.43 [e] |
| Neurocognitive summary score | Cases | −0.098 (0.064) | −0.055 (0.058) | −0.006 (0.101) | $t_1$ =0.86, p=0.39 [f] |

[a] Values given are counts (fraction of group) for association with schizophrenia and least square means (SE) for case-only associations with age of onset, WRAT-3 scores, and neurocognition.

[b] Logistic regression model with case/control status as dependent variable, rs6994992 as predictor (additive model, coded as number of copies of the minor allele T) and self-reported ancestry (Africa only, Europe only, and other) as covariate.

[c] Same model as above except rs6994992 coded as TT versus CT/CC as TT was the risk genotype for development of psychosis.

[d] Multiple regression model with age first prescribed an antipsychotic as dependent variable, rs6994992 as predictor (additive model, coded as number of copies of the minor allele T) and self-reported ancestry (Africa only, Europe only, and other) and sex as covariates. The p-value for rs6994992 was 0.69 when coded as TT versus CT/CC.

[e] Multiple regression model with WRAT-3 score at study baseline dependent variable, rs6994992 as predictor (additive model, coded as number of copies of the minor allele T) and self-reported ancestry (Africa only, Europe only, and other) and sex as covariates. The p-value for rs6994992 was 0.49 when coded as TT versus CT/CC.

[f] Multiple regression model with standardized neurocognitive composite score at study baseline dependent variable, rs6994992 as predictor (additive model, coded as number of copies of the minor allele T) and self-reported ancestry (Africa only, Europe only, and other) and sex as covariates. The p-value for rs6994992 was 0.52 when coded as TT versus CT/CC.

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